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Date of Deposit: April 27, 2005

G. Peter Nichols, Reg. No. 34,401
Name of Applicant, Assignee or Registered Representative

Signature

Our File No. 10908/8

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Chris Andre Du Plessis

Serial No. 10/756,906

Filing Date: January 14, 2004

For RECOVERY OF BIOLEACHING MICROBES

Examiner: Andrews

Group Art Unit: 1742

SUBMISSION OF CERTIFIED COPY OF PRIORITY DOCUMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Transmitted herewith is a certified copy of the following priority documents for the above-named U.S. application:

1. South African Provisional Patent Application No. 2001/5817, filed July 16, 2001; and

2. PCT/ZA02/000110 filed July 4, 2002.

Respectfully submitted,

G. Peter Nichols
Registration No. 34,401
Attorney for Applicants

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PRIORITY DOCUMENT

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Sertifikaat

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PATENT KANTOOR
DEPARTEMENT VAN HANDEL
EN NYWERHEID

Certificate

REPUBLIC OF SOUTH AFRICA

PATENT OFFICE
DEPARTMENT OF TRADE AND
INDUSTRY

Hiermee word gesertifiseer dat
This is to certify that

the documents annexed hereto are true copies of:

Application forms P.1, P2, provisional specification and drawings of South African Patent Application No. 2001/5817 as originally filed in the Republic of South Africa on 16 July 2001 in the name of BILLITON SA LIMITED for invention entitled: "RECOVERY OF BIOLEACHING MICROBES."

Geteken te **PRETORIA** in die Republiek van Suid-Afrika, hierdie **15** dag van **February 2005**

Signed at **PRETORIA** in the Republic of South Africa, this **15** day of **February 2005**

.....
Registrar of Patents

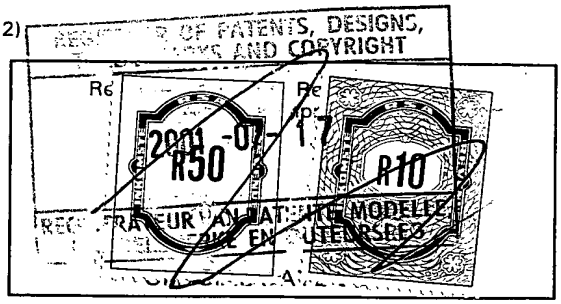
McCALLUM, RADEMEYER & FREIMOND
Ref: P.19187

FORM P1

REPUBLIC OF SOUTH AFRICA
PATENTS ACT, 1978

APPLICATION FOR A PATENT AND ACKNOWLEDGEMENT OF RECEIPT

(Section 30(1) - Regulation 22)



The grant of a patent is hereby requested by the undermentioned applicant on the basis of the present application filed in duplicate

OFFICIAL APPLICATION NO.

21	01	20015817

FULL NAME(S) OF APPLICANT(S)

71	BILLITON SA LIMITED

ADDRESS(ES) OF APPLICANT(S)

6 Hollard Street, Johannesburg, 2001

TITLE OF INVENTION

54	RECOVERY OF BIOLEACHING MICROBES

Priority is claimed as set out on the accompanying Form P2.
The earliest priority claimed is : **NONE**

This application is a patent of addition to Patent Application No.	21	01	

This application is a fresh application in terms of section 37 and based on Application No.	21	01	

THIS APPLICATION IS ACCOMPANIED BY:

X	1	A single copy of a provisional specification of .....16..... pages
	2	Two copies of a complete specification of ..... pages
X	3	...5..... sheets of Informal Drawings
	4	..... sheets of Formal Drawings
	5	Publication particulars and abstract (Form P8 in duplicate)
	6	A copy of Figure ..... of drawings (if any) for the abstract
	7	Assignment of Invention
	8	Certified priority document(s). Number(s)
	9	Translation of priority document(s)
	10	An assignment of priority rights
	11	A copy of the Form P2 and the specification of SA Patent Application 21 01
	12	A declaration and power of attorney on Form P3
	13	Request for ante-dating on Form P4
	14	Request for classification on Form P9
X	15	Form P2 in duplicate

74	ADDRESS FOR SERVICE: McCALLUM, RADEMEYER & FREIMOND, Maclyn House, June Avenue, Bordeaux P.O. Box 1130, Randburg, 2125

Dated this **16th** day of **JULY** **2001**

.....

McCALLUM, RADEMEYER & FREIMOND
PATENT AGENTS FOR APPLICANT(S)

Received — Official Date Stamp

REGISTRAR OF PATENTS, DESIGNS, TRADE MARKS AND COPYRIGHT

2001 -07- 16

REGISTRAR OF PATENTS

REGISTRATEUR VAN PATENTE, MODELLE HANDELSMERKE EN OUTEURSREG

FORM P2

M R & F Ref: P.19187

REPUBLIC OF SOUTH AFRICA — PATENTS ACT, 1978

REGISTRAR OF PATENTS

Official Application No.	Lodging date: Provisional	Acceptance date:
21 01 20015817	22 16 JULY 2001	47
International classification	**Lodging date: Complete**	**Granted date:**
51	23	

Full name(s) of applicant(s)/Patentee(s)

71 BILLITON SA LIMITED

Applicant(s) substituted:	Date Registered:
71	

Assignee(s):	Date Registered:
71	

Full name(s) of inventor(s)

72 DU PLESSIS, Chris, Andre

Priority claimed	Country		Number		Date	
Note:	33	NONE	31	NONE	32	NONE
Use International	33		31		32	
Abbreviation for Country	33		31		32	

Title of Invention:

54 RECOVERY OF BIOLEACHING MICROBES

Address of applicant(s)/patentee(s)

6 Hollard Street, Johannesburg, 2001

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74 **McCALLUM, RADEMEYER & FREIMOND, Maclyn House, June Avenue, Bordeaux, Randburg • P.O. Box 1130, Randburg 2125**

Patent of Addition to Patent No.	Date of any change:	
61		
Fresh Application based on:	**Date of any change:**	

REPUBLIC OF SOUTH AFRICA
PATENTS ACT, 1978

PROVISIONAL SPECIFICATION

(Section 30(1) - Regulation 27)

OFFICIAL APPLICATION NO			LODGING DATE	
21	01	20015817	22	16 JULY 2001

FULL NAME(S) OF APPLICANT(S)

71	BILLITON SA LIMITED

FULL NAME(S) OF INVENTOR(S)

72	DU PLESSIS, Chris, Andre

TITLE OF INVENTION

54	RECOVERY OF BIOLEACHING MICROBES

BACKGROUND OF THE INVENTION

This invention relates generally to bioleaching and more particularly is concerned with a method for recovering bioleaching microbes.

Bioleaching is the term used to refer to the microbial oxidation of reduced iron or sulphide, contained in solid mineral particles, with the subsequent liberation of valuable metals associated with these particles. Continuous culture bioleaching reactors currently in operation, commercially as well as at pilot plant scale, of which the applicant is aware, operate without separation of hydraulic retention time from solids retention time. This implies that, under certain conditions, microbial growth rates are the limiting factor in the bioleaching process. Under such conditions process benefits could be achieved by recovering microbial cells from the effluent bioleach liquor and returning these cells to the aerated bioleach reactors. The net effect of such a supplementation of recovered microbes, in high concentrations and low volumes to the bioleaching reactors, would be that the microbial accumulation of cells in these reactors would exceed that which could be achieved by microbial growth rate alone.

The activated sludge process, which is illustrated schematically in Figure 1, was developed to address water pollution problems due to a dense population and advanced industry. During wastewater treatment, organic compounds in the water are biodegraded in an aerated reactor, mainly by bacteria, resulting in the build-up of a bacterial biomass in the reactor. In order to maintain the biomass in

the aerated reactor, the inflow rate of fluid into the reactor should not exceed the rate at which the bacteria proliferate. Such a scenario would result in the washout of bacteria from the reactor with an associated loss of biodegradation capacity for the incoming organic compounds. The throughput of influent into such a reactor is therefore limited by the growth rate of the microbes in the reactor. This limitation can be overcome, however, by separating the bacterial biomass (sludge) from the rest of the liquid. This step is typically performed in a clarifier. The relatively higher density of the sludge allows for gravitational settling with an overflow of treated effluent. The concentrated sludge can then be returned to the aerated sludge reactor. This process allows for the accumulation of sludge in the aerated reactor and is referred to as activated sludge. The net effect of the process is that biomass, produced in the process of biodegrading the organic compounds contained in the influent, is not lost from the system, apart from a small amount that is purged to limit the accumulation of excess sludge. This results in a high concentration of biomass in the activated sludge reactor, which allows for a high liquid input flow rate and treatment efficiency of the reactor.

Microbial recovery of cells from bioleach bioractors is significantly more complex than the operation of a typical activated sludge plant used for wastewater treatment. In the case of wastewater treatment two phases exist, viz the biomass sludge phase (biological solids phase) and the liquid phase. In the case of bioleaching reactors three distinguishable phases exist, viz the mineral solids

particles (mineral solids), the microbial cell biomass (biological solids phase) and the liquid phase. In addition, the microbial solids phase is not as uniform as in the case of activated sludge plants. In the bioleaching scenario, biological solids are either attached onto the mineral particle surfaces or freely suspended. Separation of the biomass phase from the other phases poses significant technical difficulties and challenges. These factors are a prohibitive barrier to the implementation of cell recycling, as in the activated sludge process, to bioleaching technology.

Schiraldi et al (Reference 1) describe a microfiltration bioreactor which achieves a high cell density in *Sulfolobus solfataricus* fermentation. Use is made of a hollow fibre microfiltration unit with the main aim being to improve yield in the growth medium by removal of toxic compounds in the growth medium. Cell concentration occurs in situ in the growth reactor and there is no mention of cell recycling as in the activated sludge-type system. The medium does not contain solid mineral particles, nor is iron contained in any significant amount in the medium. The medium contains yeast extract and other organic compounds and has a pH in the range of from 3.5 to 3.8. The microfiltration unit is applied to a heterotrophic application.

The techniques described by Schiraldi et al are not useful for recovering microbial cells from bioleaching reactors. It should be borne in mind that the bioleaching environment is unique and displays the following characteristics:

(a) extreme pH conditions of 1-2;

(b) highly corrosive environments caused by the presence of high concentrations of oxidised ferric and other metals;

(c) high concentrations of ferric sulphate precipitates such as jarosite;

(d) a three phase system containing liquids (with salts in solution), biological solids phase (microbial biomass), and a minerals solids phase (precipitates, oxidised mineral particles and non-oxidised mineral particles);

(e) the exclusive presence of autotrophic microbes that do not require an organic carbon source;

(f) high temperatures (as high as 80°C) in the case of some bioleaching reactions;

(g) the fact that microbial flocculation and aggregation do not readily occur in bioleaching reactors; and

(h) the potential presence of suspended mineral solids and precipitates, together with microbial cells, in the feed solution.

SUMMARY OF THE INVENTION

The invention provides a method of recovering microbial cells in a bioleaching process which includes the steps of subjecting a slurry produced in a bioleaching

plant to a solid/liquid separation process, and extracting microbial cells from the resulting liquid.

The microbial cells may be separated from metal in solution, in the liquid.

The microbial cells may be extracted by subjecting the liquid to a centrifugal process which may be carried out continuously, or on a batch basis.

Preferably the microbial cells are extracted by subjecting the liquid to a membrane filtration process.

When use is made of the membrane filtration process the cells may be recovered by continuous concentration of the cells or the cells may be accumulated onto an inner surface of the membrane and then removed in any appropriate way for example by back flushing or washing.

The microbial cells may be extracted using a plurality of extraction phases which are operated in series.

The bioleaching plant may include at least one bioleaching reactor, and typically includes a plurality of bioleaching reactors connected in series.

The method may extend to at least one of the following steps:

(a) recycling the microbial cells to the said at least one bioleaching reactor to increase the rate of microbial cell accumulation and assist in increased bioleaching efficiency;

(b) storage and packaging of the recovered cells, including freeze-drying and cryo-preservation for future use or as a backup inoculum;

(c) inoculation of a new bioleaching reactor or re-inoculation of a currently used bioleaching reactor which may be under-performing; and

(d) extraction of bioproducts such as enzymes and proteins from the microbial biomass i.e. the extracted cells.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is further described by way of examples with reference to the accompanying drawings in which:

Figure 1 illustrates an activated sludge system (prior art) which has already been described in the preamble to this specification,

Figure 2 is a block diagram representation of a method of recovering bioleaching microbes in accordance with the principles of the invention,

Figure 3 is a more detailed illustration of the process shown in Figure 2,

Figure 4 is a diagram of a ceramic membrane microfilter for use in the methods shown in Figures 2 and 3, and

Figure 5 schematically depicts a centrifugal separation system which can be used in place of a ceramic membrane microfilter, in the method of the invention, to recover bioleaching microbes.

DESCRIPTION OF PREFERRED EMBODIMENTS

Figure 1 depicts a typical activated sludge process and has already been referred to in the preamble to this specification.

Figure 2 of the accompanying drawings is a flow chart representation of the method of the invention for recovering bioleaching microbial cells making use of a microfiltration membrane.

Bioleaching may be described as a process in which the biooxidation of ferrous iron and sulphides occurs in continuous culture agitated and aerated reactors, with the subsequent release of metals such as copper into solution[2]. In the case of gold and other precious metals, the metals of interest do not go into solution but are bio-beneficiated by the fact that the sulphides are oxidised, resulting in lower cyanide consumptions upon extraction of such metals from the remaining residue. This process can take place in a single reactor or a train of several reactors in series.

Referring to Figure 2 a concentrate feed 10 which contains a desired metal such as gold or copper, which is to be recovered, is fed to a bioleaching process 12. A slurry 14, produced by the bioleaching process, is fed to a solid/liquid separation stage 16. The oxidised minerals solids residue 18 from the separation stage 16 is treated for disposal.

The liquid or supernatant 20 from the process 16 is subjected to a cell recovery step 22 which may be effected by making use of a centrifugal technique (see Figure 5), or by making use of a membrane filtration process (see Figures 3 and 4), which separates the suspended cells 24 from the liquid 20. The recovered cells may be recycled to the process 12, be stored (block 26), be used for inoculation purposes (block 28), or be used for bioproduct production (block 30).

The metal remaining in the liquid from which the cells 24 are extracted is then recovered using a suitable process 32 which is known in the art e.g. solvent extraction or electrowinning.

Figure 3 is a more detailed depiction of the method of the invention and, where applicable, like reference numerals are used to designate like steps or components.

Referring to Figure 3 the concentrate feed 10 is fed to a bioleaching plant 12 which includes a succession of bioleaching reactors designated 12A, 12B, 12C, etc. which are connected in series. This type of process is known in the art. The slurry effluent 14 from the bioleaching chain of reactors reports to a solids/liquid separation process 16 which typically is based on the use of a clarifier or thickener 16A. During this process the minerals solids particles, together with the microbial cells which are attached to these particles, settle out from suspension under gravitation forces. A flocculent agent may be added to assist in such separation. The supernatant 20 then contains a suspension of remaining

(non-attached) microbial cells. The oxidised minerals solids residue 36 from this process is treated for disposal (step 18). The retention time in the solids/liquid separation system should be sufficiently short to ensure ongoing viability of the microbial cells. A variation of a gravitational solids/liquid separation step would be the use of a hydrocyclone or any similar process in which the gravitational selectivity and separation is induced or takes advantage of the relative density differences between the microbial cells and the minerals solids particles.

The supernatant 20 from the solids/liquid separation process, containing suspended microbial cells, is treated by using a membrane filtration process 22 to separate the suspended cells from the solution. This may be effected in one of two ways. In the first instance, shown in Figure 3, the liquid 20 is passed into a ceramic microfiltration membrane 40, which is shown in further detail in Figure 4. The membrane has a cylindrical wall 42 and is mounted inside a casing 44. The liquid 20 is pumped into an inlet 46 of the cell by means of a peristaltic pump 48, or any similar device. The pressure of the system, which is a measure of the permeability of the membrane, is monitored by means of a gauge 50.

Permeate 52 passes through the membrane wall and is collected inside the housing. The permeate flow through the membrane wall is assisted by means of a vacuum induced inside the housing, around the membrane, by means of a peristaltic pump 54 or any similar device. The level of the vacuum inside the housing is monitored by means of a gauge 56.

The permeate 52 contains metals in solution and is directed to a known metal recovery process 32, as has been described in connection with Figure 2. A flow meter 58 is used to provide a measure of the permeate flow rate from the casing 44.

The cells 24 exit the membrane tube via an outlet 60 and are continuously recovered. The rate at which the cells leave the membrane tube is measured by means of a flow meter 62.

On the input side of the membrane, i.e. at the inlet 46, the flow rate is relatively high with a relatively low cell concentration. This situation is designated in Figure 3 by means of a relatively large, low intensity, arrowhead 64. On the other hand at the outlet 60 the flow rate is relatively low but the cell concentration is relatively high, as is indicated by means of a high intensity, relatively small, arrowhead 66.

Regular membrane maintenance procedures are put in place in order to ensure sustainable efficient cross membrane flow.

Several membrane units may be used in series to improve efficiency and in order to allow for sequential and automated back-flushing and membrane maintenance procedures to be implemented. In the case of thermophilic microbial cells, the membrane reactor, the solids/liquid separation system, and the solution entering and exiting the membrane reactor should preferably be maintained at a temperature sufficiently high to maintain metabolic activity of these cells.

As has been described in connection with Figures 2 and 3 the recovered concentrated cell suspension 24 could be used for any, or a combination, of the following:

(a) recycled to primary or secondary bioleaching reactors in order to increase the rate of microbial cell accumulation and assist in increased bioleaching efficiency;

(b) storage and packaging, including freeze drying and cryo-preservation for future use or as backup inoculum;

(c) inoculation of new reactors or re-inoculation of currently used reactors that may be under performing; and

(d) extraction of enzymes, proteins or other bioproducts from microbial biomass.

The process of the invention could also be applied in a system where a step is introduced to remove attached microbial cells from solid mineral particles.

The following bioleaching process benefits can be achieved directly or indirectly from the recovery and recycling of cells to, and from, a bioleaching reactor plant:

(a) increased cell concentrations in the reactors;

(b) higher minerals oxidation rates as a result of increased cell numbers;

(c) control of the microbial concentration density in different reactors by selective addition to different reactors;

(d) increased process robustness. The higher cell concentrations would be less effected by process upsets and inhibitory conditions than would be the case for reactors with lower cell concentrations;

(e) any microbial adaptations that occur in the reactors would be retained and recycled. This would result in the eventual proliferation of any such mutated or adapted organism if such an adaptation provided the particular cell with a competitive advantage under the prevailing reactor conditions; and

(f) cells with various growth rate kinetics could be accommodated in the reactors. In continuous culture reactor systems where the hydraulic retention time is identical to the solids retention time and the biological sludge retention time, the microbial growth rate is controlled by the hydraulic retention time. In such a reactor, at steady state, the microbial growth rate of the cells is equal to the dilution rate (reciprocal of the hydraulic retention time). Microbes with a maximum specific growth rate less than the prevailing dilution rate would, therefore, be accommodated in such a reactor. Microbes with a maximum specific growth rate greater than the prevailing dilution rate would, however, be lost (washed out) from the reactor even if such microbes have superior affinity for ferrous and/or

sulphide (smaller half saturation constants for these substrates) or have other superior qualities beneficial in the bioleaching process. Discrimination in the continuous culture process is, therefore, mainly based on growth rates at the prevailing conditions. By recovering and recycling the cells to the reactors, the microbial cell (biological sludge) retention time is separated from the hydraulic and mineral solids retention times. This allows for the accommodation of microbes with a greater range of maximum specific growth rates and, therefore, also of microbes with other beneficial bioleaching properties.

Effective monitoring of the membrane recovery system could include the following elements:

(a) monitoring of viability (activity) of microbial cells by determining their activity prior to and after membrane recovery using respirometry (Micro-Oxymax) methods (oxygen and carbon dioxide consumption) or ferrous oxidation capacity;

(b) monitoring of microbial cell numbers by direct microscopic counting, particle size analyses (Cell Facts) and protein concentration determinations;

(c) monitoring of the cross-membrane flux by measuring the balance of flow rates of the influent, effluent and permeates respectively; and

(d) monitoring of the cross-membrane pressure drops by measuring the inlet pressure and vacuum pressure applied to obtain a specific flow rate across the membrane.

Example

The process described hereinbefore has been tested by using equipment with the following specifications:

- Membrane type: Ceramic microfiltration membrane
- Length: 0.212 m
- Diameter: 0.012 m
- Module setup: 3 membranes in series
- Membrane area: 0.024 m^2 per module
- Membrane wall thickness: 0.7 mm

Summary of Results

The system was operated using two regimes: vacuum assisted permeate removal and constant flux operation. Both operating regimes reached and exceeded a 50-fold concentration factor in a single pass objective, at a flux of 56ℓ m^{-2} h^{-1} and 99% biomass retention. This far exceeds the performance of commercial systems, in which a 20-fold concentration is typically achieved at fluxes of $20 - 40\ell\ m^{-2}\ h^{-1}$.

Figure 5 schematically illustrates a centrifugal based system which can be used in place of the membrane microfilter shown in Figures 3 and 4.

The supernatant 20 is directed into a centrifuge 70 which is spun at high speed and which separates the microbial cells from the remaining solution in the centrifuge. The cells 24 are drawn from the centrifuge and are used in any of the

applications which have been described. Solution which is depleted of cells is treated (step 32) for metal recovery e.g. by means of solvent extraction or electrowinning.

The centrifugal separation system can be operated on a batch or continuous basis to achieve results which are similar to those obtained by means of the membrane filter.

References

1. Schiraldi C, Marulli F, Di Lernaia I, Martino A, De Rosa M. 1999. A microfiltration bioreactor to achieve high cell density in *Sulfolobus solfataricus* fermentation. Extremophiles 3, 199-204.

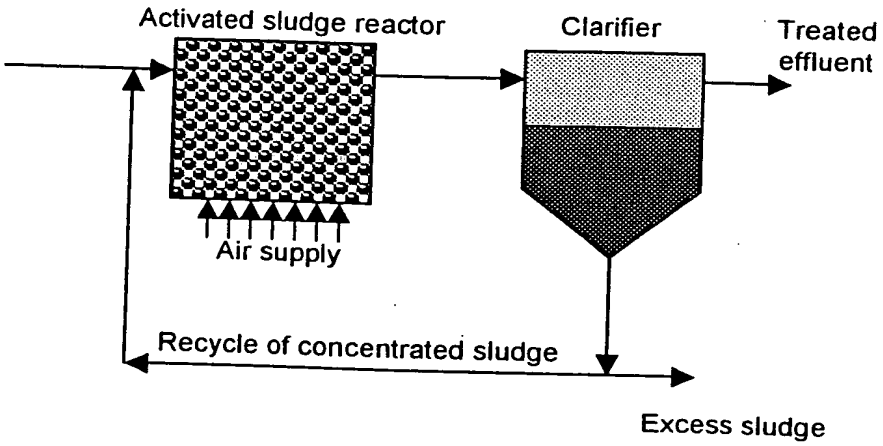
2. MIRO, The use of micro-organisms in the minerals and metals industries, Technical Review Series No 6., Vol 3, 1995-2000. Minerals Industry Research Organisation, Lichfield UK. ISBN 1 872440 22 3.

DATED this 16th day of JULY 2001

McCALLUM RADEMEYER & FREIMOND
Patent Agents for the Applicant

P.19187/bjt

Figure 1: Traditional Activated Sludge System



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Patent Agents for the Applicant

Recovery of Bioleaching Microbes

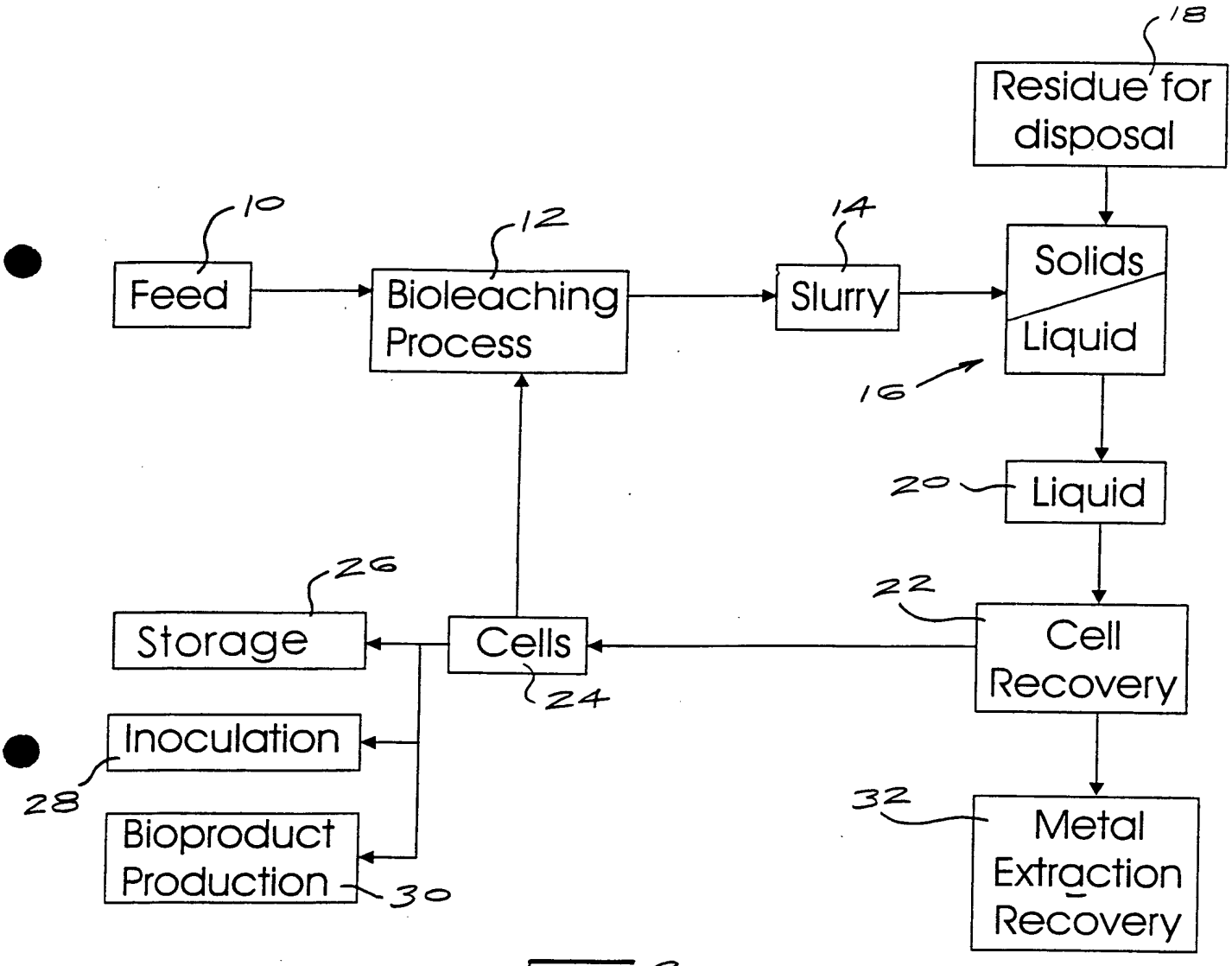

FIG 2

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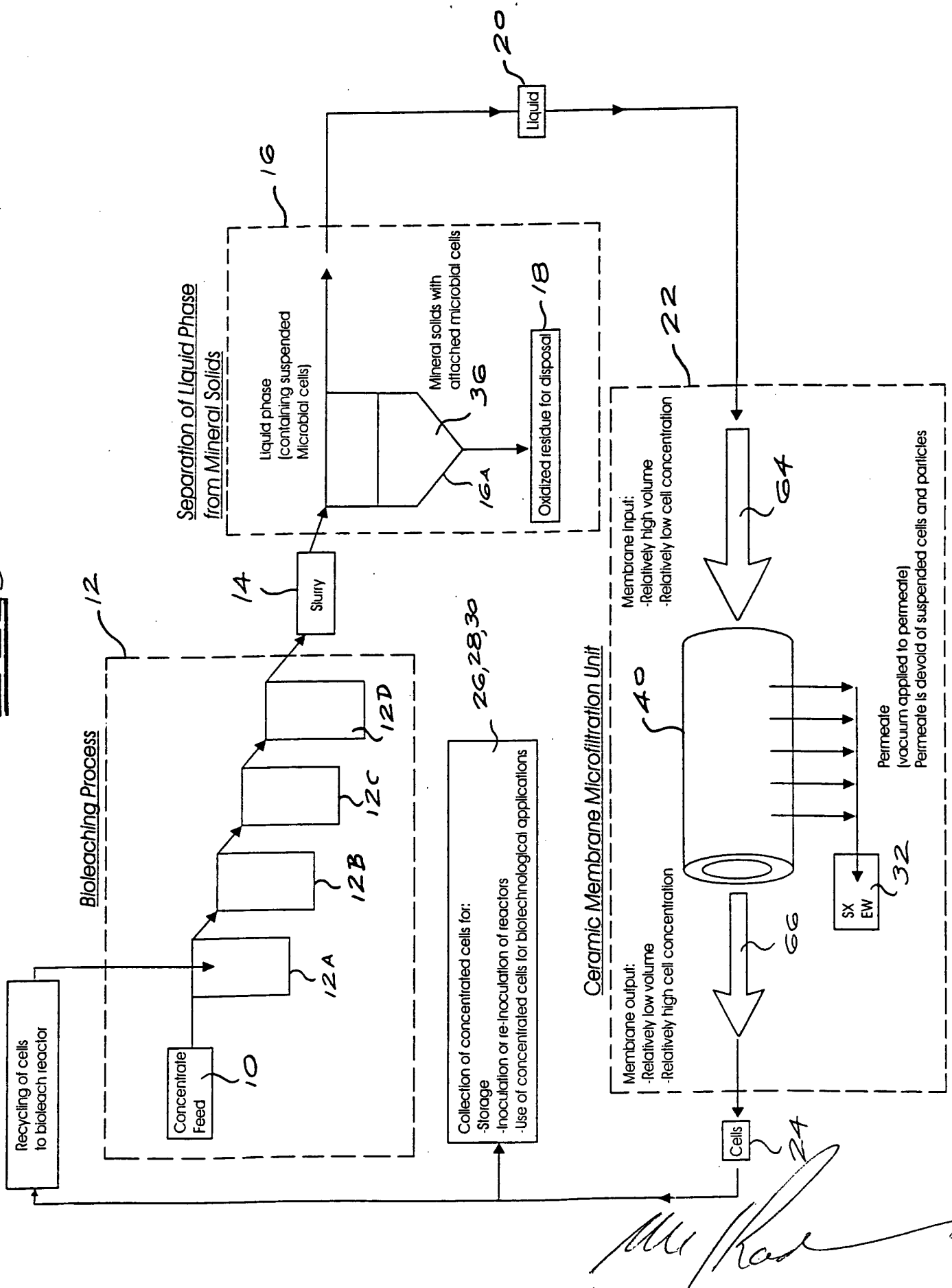

Fig 3

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Diagram of Ceramic Membrane Reactor Microfiltration Process

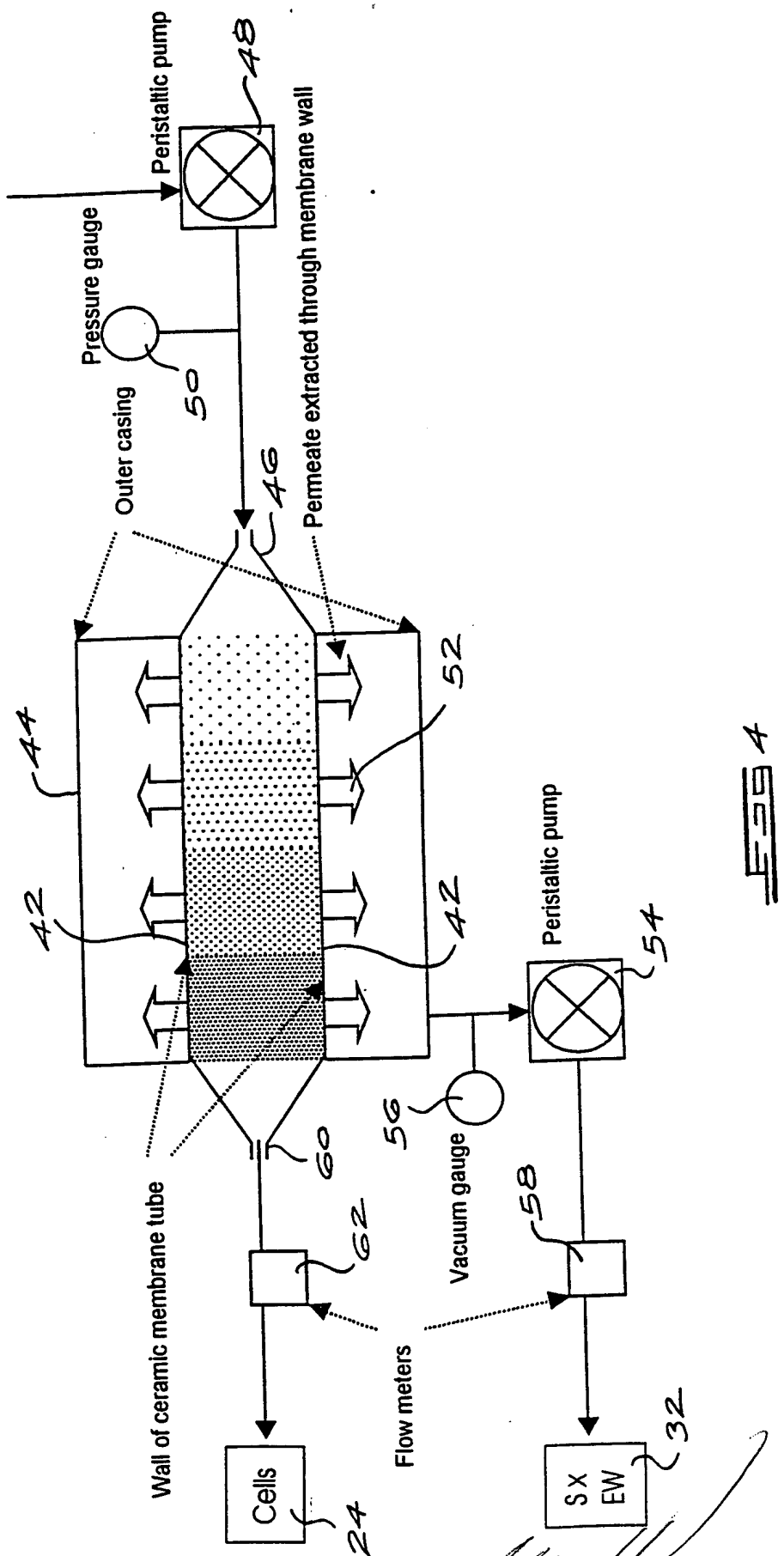

Fig 4

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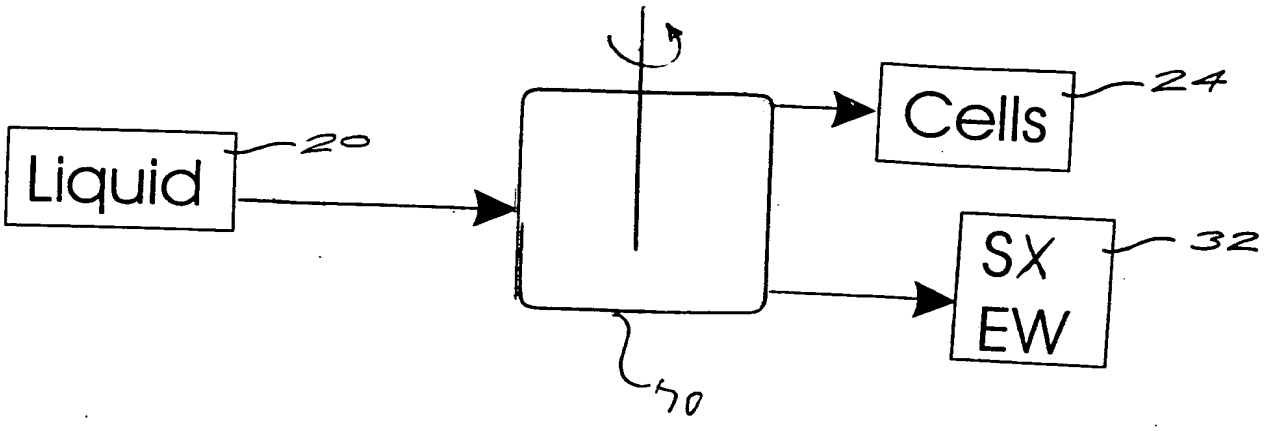

Fig 5

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